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Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L15	L14 and (termin$3 or end$1)	29	L15
L14	L13 and (composition$1 or kit$1)	29	L14
L13	L12 and ribonucleotide$1	31	L13
L12	l1 and polymerase$1 and reverse transcriptase$1	230	L12
L11	l1 and polymerase$1	362	L11
L10	l9 and reverse transcriptase$1	0	L10
L9	L8 and (composition$1 or kit$1)	6	L9
L8	L7 and modif$3	6	L8
L7	l4 and reverse	6	L7
L6	l4 and polymerase$1	0	L6
L5	L4 and polymerase and reverse	0	L5
L4	L3 and (termin$3 or end$1)	6	L4
L3	L2 and (composition$1 or kit$1)	6	L3
L2	(oligonucleotide$1 or primer$1 or probe$1) near5 alkyl ribonucleotide$1	6	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	(oligonucleotide or primer or probe$1) near5 alkyl	787	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 10 of 29 returned.

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Term	Documents
COMPOSITION$1	0
COMPOSITION	1472350
COMPOSITIONA	11
COMPOSITIONB	3
COMPOSITIONC	1
COMPOSITIOND	5
COMPOSITIONE	3
COMPOSITIONF	2
COMPOSITIONG	10
COMPOSITIONH	1
COMPOSITIONL	5
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Previous Page Next Page

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L15: Entry 17 of 29 File: USPT Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6130038 A
TITLE: Method for amplifying target nucleic acids using modified primers

Abstract Text (1):
The present invention concerns oligonucleotides containing one or more modified nucleotides which increase the binding affinity of the oligonucleotides to target nucleic acids having a complementary nucleotide base sequence. These modified oligonucleotides hybridize to the target sequence at a faster rate than unmodified oligonucleotides having an identical nucleotide base sequence. Such modified oligonucleotides include oligonucleotides containing at least one 2'-O-methylribofuranosyl moiety joined to a nitrogenous base. Oligonucleotides can be modified in accordance with the present invention to preferentially bind RNA targets. The present invention also concerns methods of using these modified oligonucleotides and kits containing the same.

Brief Summary Text (2):
This invention pertains to methods and compositions for detecting and amplifying nucleic acid sequences using oligonucleotides which contain one or more nucleotides having a modification or modifications resulting in increased target affinity. Such oligonucleotides have been unexpectedly discovered to hybridize to a target nucleic acid at a significantly greater rate than a corresponding unmodified oligonucleotide hybridizes to the same target. As a result, the methods and compositions of the present invention offer advantages for applications employing nucleic acid hybridization, such as medical and veterinary diagnostics, food testing and forensics.

Brief Summary Text (5):
Most nucleic acid hybridization schemes have features in common. One such typical feature is the use of single-stranded nucleic acid probes (or denatured double-stranded probes) having a defined or known nucleotide sequence. Probe molecules may be derived from biological sources, such as genomic DNA or RNA, or may be enzymatically synthesized, either in a prokaryotic or eukaryotic host cell or in vitro. Presently, most nucleic acid probes in common use are oligonucleotide probes made using chemical synthetic methods ("synthetic oligonucleotides"). One such synthetic method is automated sequential addition of 3'-activated, protected nucleotides to the 5' end of a growing, solid phase-bound oligonucleotide chain, followed by cleavage of the completed oligonucleotide from the support and deprotection. See, e.g., Eckstein, Oligonucleotides & Analogues: A Practical Approach (1991).

Brief Summary Text (8):
By "complementary" is meant that the nucleotide sequences of corresponding regions of two single-stranded nucleic acids, or two different regions of the same single-stranded nucleic acid, have a nucleotide base composition that allows the single strands to hybridize together in a stable double-stranded hydrogen-bonded region under stringent hybridization conditions. When a contiguous sequence of nucleotides of one single stranded region is able to form a series of "canonical" hydrogen-bonded base pairs with an analogous sequence of nucleotides of the other single-stranded region, such that A is paired with U or T, and C is paired with G, the nucleotides sequences are "perfectly" complementary.

Brief Summary Text (9):
The extreme specificity of nucleic acid hybridization, which under some circumstances can allow the discrimination of nucleic acids differing by as little as one base, has allowed the development of hybridization-based assays of samples containing specific microorganisms, nucleic acids bearing given genetic markers, tissue, biological fluids

and the like. Such assays are often able to identify nucleic acids belonging to particular species of microorganisms in a sample containing other, closely-related species. Nucleic acid hybridization assays can also specifically detect or identify certain individuals, or groups of individuals, within a species, such as in the forensic use of RFLP (restriction fragment length polymorphism) and PCR (polymerase chain reaction) testing of samples of human origin.

Brief Summary Text (25):
This invention concerns diagnostic methods and compositions employing nucleic acid hybridization techniques. Applicant has surprisingly discovered that oligonucleotides, comprised of one or more modified nucleotides, which have increased binding affinity to a target nucleic acid having a complementary nucleotide sequence, will hybridize to the target nucleic acid at a faster rate than unmodified oligonucleotides. The inventions described herein are drawn to the use of oligonucleotides, wholly or partially so modified, in methods involving their use as, for example, hybridization assay probes, amplification primers, helper oligonucleotides, and oligonucleotides for the capture and immobilization of desired nucleic acids.

Brief Summary Text (29):
Because the T.sub.m of the modified oligonucleotides is higher than that of corresponding, unmodified oligonucleotides of the same base sequence, the compositions and diagnostic methods described herein enable the use of oligonucleotides and oligonucleotide probes of shorter length than are otherwise practical for the specific hybridization and detection of nucleic acid targets (preferably RNA targets). The use of shorter oligonucleotides to specifically bind to target nucleic acids at a given temperature has additional advantages. For instance, shorter oligonucleotides will generally have a greater ability to discriminate perfectly complementary targets from "mismatched" base sequence regions. Shorter oligonucleotides are also less likely to overlap undesirable base sequences. Additionally, because of the higher T.sub.m, the modified oligonucleotides can stably hybridize at higher temperatures than their unmodified counterparts.

Detailed Description Text (5):
By "synthesizing" a nucleic acid or oligonucleotide is meant making the nucleic acid by chemical synthesis or enzymatic means. It is known that certain nucleic acid polymerase enzymes can incorporate modified nucleotides during enzymatic synthesis.

Detailed Description Text (6):
By "modified", a "modified nucleotide" or "modification" is meant a purposeful variant from the classical ribo- and deoxyribonucleotides A, T, G, C and U. When used in this specification, modified will mean a variant of the classical nucleotides, said variants leading to a higher binding efficiency when an oligonucleotide which contains said modified nucleotides is hybridized to a target nucleic acid than when the same oligonucleotide contains the classical nucleotides. In some cases an oligonucleotide having a modified 3' end may be referred to. This means

Detailed Description Text (7):
that the 3' end of the oligonucleotide contains a substitution which inhibits or prevents extension of the 3' end by a nucleic acid polymerase.

Detailed Description Text (13):
The methods and compositions of the present invention result from the unexpected discovery that oligonucleotides containing one or more nucleotides modified so that the oligonucleotides have an increased T.sub.m for a given target (as compared to otherwise identical unmodified oligonucleotides) will hybridize to a given target at an increased rate as compared to unmodified oligonucleotides. A maximum increase in the hybridization rate of a modified oligonucleotide occurs when a "cluster" of nucleotides are modified. By "cluster" is meant that at least about 4 of 5 consecutive nucleotides are so modified. Thus, oligonucleotides containing a mixture of modified and unmodified nucleotides may be just as effective in increasing target hybridization rate as in oligonucleotides containing 100% modified nucleotides. Aspects of the invention feature "chimeric" oligonucleotides containing both modified and unmodified nucleotides.

Detailed Description Text (15):

Thus, the present invention is directed to diagnostic methods and compositions involving modified oligonucleotides which display an increase in the rate of oligo:target hybridization over an unmodified oligonucleotide of the same base sequence.

Detailed Description Text (26):
Therefore, one aspect of the present invention provides a means for increasing hybridization rates, as well as binding affinity, of oligonucleotides for RNA targets by using oligonucleotides containing nucleotides having a substitution at the 2' position of the ribofuranosyl ring ("2'-modified oligonucleotide"); preferably an alkoxy substitution, most preferably a methoxy substitution. These properties render useful methods employing such oligonucleotides in a diagnostic hybridization assay format by increasing the rate and extent of hybridization of such an oligonucleotide without requiring a concomitant increase in the concentrations of the hybridizing nucleic acids, a change in the properties or composition of the hybridization solution, the addition of "helper oligonucleotides", disclosed in Hogan & Milliman, U.S. Pat. No. 5,030,557, or an increase in the hybridization temperature. U.S. Pat. No. 5,030,557 enjoys common ownership with the present invention and is incorporated by reference herein. Nonetheless, the methods of the present invention may be used as supplements to one or more of these other techniques in any procedure in which an increase in the rate of nucleic acid hybridization would be advantageous.

Detailed Description Text (46):
In still other aspects, the present invention includes methods for employing modified oligonucleotide primers, promoter-primers, and/or splice templates for nucleic acid amplification and compositions comprising such oligonucleotides, wherein the oligonucleotides contain at least one cluster of modified bases which cause an increased rate of hybridization.

Detailed Description Text (47):
Primer-employing amplification methods include the polymerase chain reaction method (PCR) and its variations, as described by Mullis, et al., (see U.S. Pat. Nos. 4,683,195, 4,683,202, and 4,800,159, European Patent Application Nos. 86302298.4, 86302299.2, and 87300203.4, and 155 Methods in Enzymology, 335-350 (1987)). The PCR methodology is by now a matter of common knowledge to those skilled in the art.

Detailed Description Text (50):
Each of these methods makes use of one or more oligonucleotide primers or splice templates able to hybridize to or near a given nucleotide sequence of interest. After hybridization of the primer, the target-complementary nucleic acid strand is enzymatically synthesized, either by extension of the 3' end of the primer or by transcription, using a promoter-primer or a splice template. In some amplification methods, such as PCR, rounds of primer extension by a nucleic acid polymerizing enzyme is alternated with thermal denaturation of complementary nucleic acid strands. Other methods, such as those of Kacian & Fultz, supra, McDonough, et al., supra, and Kacian, et al., supra, are isothermal transcription-based amplification methods.

Detailed Description Text (61):
Other immobilization methods may include the use of a linker arm, for example, N-hydroxysuccinamide (NHS) and its derivatives, to join the oligonucleotide to the solid support. Common solid supports in such methods are, without limitation, silica, polyacrylamide derivatives and metallic substances. In such a method, one end of the linker may contain a reactive group (such as an amide group) which forms a covalent bond with the solid support, while the other end of the linker contains another reactive group which can bond with the oligonucleotide to be immobilized. In a particularly preferred embodiment, the oligonucleotide will form a bond with the linker at its 3' end. The linker is preferably substantially a straight-chain hydrocarbon which positions the immobilized oligonucleotide at some distance from the surface of the solid support. However, non-covalent linkages, such as chelation or antigen-antibody complexes, may be used to join the oligonucleotide to the solid support.

Detailed Description Text (67):
Another option combines the elements of detection and nucleic acid amplification. In such a system, the target nucleic acid is immobilized as described, for example, and

without limitation, in the assay procedures described above. One or more amplification oligonucleotides (see, e.g., Kacian, et al., WO93/22461), such as a primer, promoter-primer, or splice template, able to hybridize with a specific region of the target nucleic acid may be contacted with the immobilized target nucleic acid under nucleic acid amplification conditions, e.g., in the presence of one or more nucleic acid polymerases and ribo- and/or deoxyribonucleotide triphosphates.

Detailed Description Text (71):
Applicant prefers to use the transcription-based amplification method described in Kacian & Fultz, supra, previously incorporated by reference. In accord with this method, a promoter-primer having a 3' region complementary to a portion of the target and a 5' region and a primer having the same nucleotide sequence as a portion of the target are contacted with a target RNA molecule. The primer and promoter-primer define the boundaries of the target region to be amplified, including both the sense present on the target molecule and its complement, and thus the length and sequence of the amplicon. In this preferred embodiment, the amplification oligonucleotides and immobilized target RNA are contacted in the presence of effective amounts of Moloney murine leukemia virus-derived reverse transcriptase and T7 RNA polymerase, both ribonucleotide and deoxyribonucleotide triphosphates, and necessary salts and cofactors at 42.degree. C. Under these conditions, nucleic acid amplification occurs, resulting predominantly in production of RNA amplicons of a sense opposite to that of the target nucleic acid. These amplicons are then detected, e.g., by using an acridinium ester-labeled hybridization assay probe of the same sense as the target-nucleic acid, in the hybridization protection assay disclosed in Arnold, supra, previously incorporated by reference.

Detailed Description Text (72):
In this preferred embodiment, Applicant prefers that the 3' terminus of the immobilized oligonucleotide, target capture oligonucleotide and coupling oligonucleotide(s) be "capped" or blocked to prevent or inhibit their use as templates for nucleic acid polymerase activity. Capping may involve addition of 3' deoxyribonucleotides (such as cordycepin), 3', 2'-dideoxynucleotide residues, non-nucleotide linkers, such as disclosed in Arnold, et al., supra, alkane-diol modifications, or non-complementary nucleotide residues at the 3' terminus.

Detailed Description Text (75):
It will be clear to the skilled person that this methodology is amenable, either as described or with obvious modifications, to various other amplification schemes including the polymerase chain reaction.

Detailed Description Text (81):
In light of the present disclosure, those of skill in the art will easily recognize that using modified helper oligonucleotides which will hybridize with the target nucleic acid at an increased rate over their unmodified counterparts can lead to even greater hybridization rates of the labeled probe to their target. Thus, methods and compositions for detecting oligonucleotides employing such modified helper oligonucleotides are intended to be encompassed within the scope of this invention. Preferred helper oligonucleotides have modifications which give them a greater avidity towards RNA than DNA. In a preferred embodiment, such modifications include a cluster of at least about 4 2'-O-methyl nucleotides. In a particularly preferred embodiment, such modifications would include a cluster of about 8 2'-O-methyl nucleotides.

Detailed Description Text (82):
Diagnostic Kits

Detailed Description Text (83):
The methods described herein also clearly suggest diagnostic kits specially formulated for use in such methods. These kits will contain one or more oligonucleotides to be used in a diagnostic nucleic acid hybridization assay. At least one of these oligonucleotides will contain a cluster of at least about 4 modified nucleotides designed to hybridize to a target nucleic acid region at an increased rate over an otherwise identical oligonucleotide.

Detailed Description Text (84):
Such diagnostic kits may include, without limitation, one or any combination of the

probe, amplification, helper and sample processing oligonucleotides described herein.

Detailed Description Text (85):
In a preferred embodiment of the present invention, the kit contains at least one labeled oligonucleotide probe having a region containing one or more clusters of at least about 4 contiguous 2'-modified nucleotide residues. In a more preferred embodiment, the region contains one or more clusters of about 8 2'-modified nucleotides.

Detailed Description Text (87):
Kits containing one or more of the modified oligonucleotides disclosed herein could be sold for use in any diagnostic hybridization assay method, or related amplification method, of the present invention. In such an assay, at least one of the modified oligonucleotides contained in the kit would function as a probe able to hybridize to a target nucleic acid. If the modified probe is contacted with a sample containing the target nucleic acid, the probe will exhibit improved hybridization properties over an unmodified probe having an identical base sequence. For instance, the hybridization binding affinity between the target and the probe will be greater than the hybridization binding affinity between the target and an unmodified form of the probe, when subjected to the same hybridization assay conditions. Additionally, the hybridization rate between the target and the probe will be greater than the hybridization rate between the target and an unmodified form of the probe, when subjected to the same hybridization assay conditions.

Detailed Description Text (88):
To further improve the hybridization properties of the probe, one or more conjugate molecules may bound to the probe, preferably in a region containing a cluster of at least about 4 modified nucleotides. It is also expected that the kit would be packaged with instructions for using one or more modified oligonucleotides in a diagnostic hybridization assay of the present invention.

Detailed Description Text (96):
It is a further object of the present invention to provide kits including one or more oligonucleotides containing modified nucleotides which function to increase the rate of hybridization between the oligonucleotide and a target nucleic acid. Kits of the present invention could include any combination of probe, amplification, helper and sample processing oligonucleotides. In a preferred embodiment, the modified oligonucleotides of these kits would contain at least one cluster of about 4 2'-O-methyl modifications to the ribofuranosyl ring. Kits containing these modified oligonucleotides may be supplied for use in both diagnostic hybridization assays and amplification assays. Such kits may further include written instructions directing practitioners in the use of the modified oligonucleotides in either or both diagnostic hybridization assays or amplification assays.

Detailed Description Text (98):
Given the present disclosure, it will be understood that certain embodiments of the methods and compositions, including the kits, of the present invention may employ oligonucleotides having more than one type of modification affecting the hybridization properties of the resulting oligonucleotide, i.e., T.sub.m and hybridization kinetics. Such multiple modifications may act in a cooperative fashion to further increase the hybridization rate or to increase the specificity of the resulting oligonucleotide for a given type of nucleic acid target, such as RNA. Furthermore, chimeric oligonucleotides may have or consist of regions of differently modified oligonucleotides containing either 2'-modified nucleotides or nucleotides having other modifications or both.

Detailed Description Text (99):
The objects and aspects of the invention specifically described herein are not intended as an exhaustive listing of the objects or aspects of the methods and compositions of the present invention which would be apparent to those skilled in the art in light of the present disclosure. Nor should the preceding description or the Examples which follow be construed as limiting the invention to the embodiments specifically disclosed therein.

Detailed Description Text (103):

In these Examples, the acridinium esters were attached, using standard chemical techniques, to a non-nucleotide monomeric unit having a primary amine "linker arm" joined to the acridinium ester moiety, which is inserted between contiguous sequences of nucleotides during the chemical synthesis of the oligonucleotides, or placed at a terminal position of the oligonucleotide. See, Arnold, et al., Non-Nucleotide Linking Reagents for Nucleotide Probes, EPO Publication No. EPO 313219, which enjoys common ownership with the present invention, and is now incorporated by reference herein. However, it will be understood that the preference of 2'-modified oligonucleotides for RNA targets and the effect of the modified oligonucleotides on the rate of hybridization to DNA targets are not determined by the presence or specific nature of a label. Thus, those of skill in the art will recognize that oligonucleotides used in the methods of the present invention may be labeled with a variety of labels, or they may be unlabelled when, for example, they are used as amplification primers, helper oligonucleotides or in a capture assay.

Detailed Description Text (106):
It will nevertheless be clear to those of skill in the art, in light of the present disclosure, that other labels may be used in the methods and compositions of the present invention without departing from the spirit of the invention disclosed herein.

Detailed Description Text (110):
The probes, as illustrated below, were synthesized to contain no 2'-O-methyl nucleotides (Probe A), all 2'-O-methyl nucleotides (Probe B), or a combination of deoxy- and 2'-O-methyl nucleotides (Probes C, D and E). Probe C contained four contiguous deoxyribonucleotides positioned directly adjacent to each side of the linker attachment site and 2'-O-methyl ribonucleotides at all other bases; Probe D contained four contiguous 2'-O-methyl nucleotides positioned directly adjacent to each side of the linker attachment site and deoxyribonucleotides at all other bases, and Probe E contained eight contiguous 2'-O-methyl nucleotides positioned directly adjacent to each side of the linker attachment site and deoxyribonucleotides at all other bases. The T.sub.m of each hybrid was determined using both a chemiluminescent and an optical method.

Detailed Description Text (131):
To compare the relative stabilities of hybrids containing various combinations of DNA, RNA, and 2'-O-methyl nucleotide strands, acridinium ester-labeled oligonucleotide probes of SEQ ID NO: 1 (see Example 1 above) were hybridized to synthetic targets having a perfectly complementary base sequence. The probes and target sequences contained 100% ribonucleotides (RNA), 100% deoxyribonucleotides (DNA) or 100% 2'-O-methyl nucleotides in the combinations indicated in Table 5. The melting characteristics of each tested hybrid, as determined either using the chemiluminescent or the optical method, is shown in Table 5 below. More than one data point in the table indicates an independent, duplicate experiment.

Detailed Description Text (153):
A summary of the relative hybridization rates of an acridinium ester-labeled probe consisting entirely of deoxy- or 2'-O-methyl ribonucleotides determined by these four methods is summarized in Table 9. The data resulting from these experiments indicate that at 60.degree. C. an oligonucleotide consisting entirely of 2'-O-methyl nucleotides hybridizes 2.3-fold faster than the corresponding deoxyribonucleotide probe.

Detailed Description Text (208):
As summarized in Table 23 below, at 70.degree. C. and 80.degree. C. acridinium ester-labeled probes containing 2'-O-methyl nucleotides exhibited DH ratios comparable to acridinium ester-labeled probes containing deoxyribonucleotides at 60.degree. C. Thus, elevated temperature may be used in diagnostic assays employing the methods and compositions of the present invention without a detectable decrease in assay sensitivity due to degradation of the label.

Detailed Description Text (220):
In order to illustrate the general usefulness of the compositions and methods of the present invention in the diagnostic application of nucleic acid hybridization technology, oligonucleotides were constructed having a modification other than a

2'-modification to the ribofuranosyl moiety, but which also caused an increase in the binding affinity of a probe for its target. In this example, oligonucleotides were synthesized containing two nucleotides modified at the nitrogenous base. Specifically, N-diisobutylaminomethylidene-5-(1-propynyl)-2'-deoxycytidine; (a cytidine analog) and 5-(1-propynyl)-2'-deoxyuridine) (a thymidine analog). These nucleotide analogs are commercially available, for example, from Glen Research in Sterling, Va.

Detailed Description Paragraph Table (23):
TABLE 21 Probe Ribonucleotides t1/2 (Probe) t1/2 (Hybrid) DH A A deoxy .81 49.1 60.3 B 2'-O-methyl .63 77.2 123.5 B F deoxy .36 20.79 7.74 F 2'-O-methyl .89 75.25 4.55 C H deoxy .69 17.26 25 H 2'-O-methyl .76 44.7 58.8 D G deoxy .62 25.67 41.4 G 2'-O-methyl .81 23.55 29.7

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Keller et al., "Synthesis and hybridization properties of oligonucleotides containing 2'-O-modified ribonucleotides," Nucl. Acid. Res., 21(19):4499-4505 (1993).

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Inoue et al., "Synthesis and hybridization studies on two complementary nona(2'-O-methyl)ribonucleotides", Nucleic Acids Research, 15(15):6131-6148 (1987).

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Iribarren et al., "2'-O-Alkyl oligoribonucleotides as antisense probes", Proc. Natl. Acad. Sci, USA, 87:7747-7751 (1990).

Other Reference Publication (45):
Yamaguchi et al., "Chemical synthesis of the 5'-terminal part bearing cap structure of messenger RNA of cytoplasmic polyhedrosis virus (CPV): m.sup.7 G.sup.5, pppAmpG and m.sup.7 G.sup.5, pppAmpGpU", Nucleic Acids Res., 12(6):2939-2954 (1984).

CLAIMS:

1. A method for amplifying a target nucleic acid sequence contained in a target nucleic acid analyte, said method comprising the steps of:

a) contacting a sample suspected of containing said target analyte with an oligonucleotide primer under conditions such that a first nucleotide base region of said primer forms a stable hybrid with a second nucleotide base region of said target analyte, wherein said first nucleotide base region contains one or more ribonucleotides modified to include a 2'-O-methyl substitution to the ribofuranosyl moiety; and

b) incubating said sample under conditions such that said target sequence is amplified.

2. The method of claim 1, wherein said first nucleotide region of said primer includes one or more clusters of at least 4 of said modified ribonucleotides.

3. The method of claim 1, wherein said first nucleotide region of said primer includes one or more clusters of at least 6 of said modified ribonucleotides.

4. The method of claim 1, wherein said first nucleotide region of said primer includes one or more clusters of at least 8 of said modified ribonucleotides.

5. The method of claim 1, wherein each nucleotide of said primer is a ribonucleotide modified to include a 2'-O-methyl substitution to the ribofuranosyl moiety.

8. The method of claim 1 further comprising contacting said sample with nucleoside triphosphates and at least one nucleic acid polymerase.

9. The method of claim 8, wherein said nucleoside triphosphates are added to the 3' terminus of said primer by said polymerase.

10. The method of any one of claims 1 to 5 further comprising contacting said sample

with a nuclease inhibitor, said nuclease inhibitor being other than a polynucleotide containing a ribonucleotide modified to include a 2'-O-methyl substitution to the ribofuranosyl moiety.

13. The method of any one of claims 1 to 5, wherein said target sequence is amplified using a polymerase chain reaction method of amplification.

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Generate Collection

L15: Entry 9 of 29 File: USPT Aug 27, 2002

DOCUMENT-IDENTIFIER: US 6440723 B1
TITLE: Arrays with modified oligonucleotide and polynucleotide compositions

Brief Summary Text (8):
The present invention provides arrays having associated polymer sequences which are preferably oligonucleotide and/or polynucleotide polymers with modified structures (e.g., 1', 2', 3', 5' and/or modifying the ribose oxygen), methods of making such arrays, assays for using such arrays, and kits containing such arrays. The arrays of the present invention are attached to the substrate surface via a non-covalent linkage, e.g., a modified polynucleotide having a biotin group is attached to a substrate having a surface coated with avidin. The modifications described herein provide numerous advantages, including ease and efficiency of manufacturing probes with a higher binding affinity for complementary nucleic acids, acid resistance, and/or nuclease resistance.

Brief Summary Text (14):
In yet another embodiment, the modified associated oligonucleotide and/or polynucleotide polymers of the array exhibit substantial resistance to nuclease degradation. These molecules preferably have an end-blocking group that confers nuclease resistance to the molecule at one or both ends of the molecule, and preferably at least one of the end blocks is also a binding unit, e.g., biotin or a biotin analog.

Brief Summary Text (15):
It is thus an object of the invention to provide arrays having associated chemically modified oligonucleotide and/or polynucleotide polymers to confer substantial nuclease resistance. Nucleases can be used to digest the test substrate binding agent, freeing the associated binding agents for further use. The location of the chemical modification can be determined depending on the binding of the polymer to the substrate and/or the desired nuclease used with the array (e.g., an array to be treated with a 3' exonuclease can have a 3' end blocking group on the polymers). The associated oligonucleotides and/or polynucleotides remain unaffected as to their binding capacity with test nucleic acids.

Brief Summary Text (21):
It is another advantage that the modified oligonucleotide and/or polynucleotide polymers of the array may be synthesized to have approximately the same T.sub.m, by varying the length of the modified polymers. Thus, modified polymers will have the same T.sub.m between compositions allowing for better control of hybridization.

Drawing Description Text (3):
FIGS. 8-9 illustrate the chemical structure of end-blocked, acid stable molecules used in the invention.

Detailed Description Text (9):
The terms "nucleic acid" and "nucleic acid molecule" as used interchangeably herein, refer to a molecule comprised of one or more nucleotides, i.e., ribonucleotides, deoxyribonucleotides, or both. The term includes monomers and polymers of ribonucleotides and deoxyribonucleotides, with the ribonucleotides and/or deoxyribonucleotides being connected together, in the case of the polymers, via 5' to 3' linkages. However, linkages may include any of the linkages known in the nucleic acid synthesis art including, for example, nucleic acids comprising 5' to 2' linkages. The nucleotides used in the nucleic acid molecule may be naturally occurring or may be synthetically produced analogues that are capable of forming base-pair relationships with naturally occurring base pairs. Examples of non-naturally occurring bases that are capable of forming base-pairing relationships include, but are not limited to, aza

and deaza pyrimidine analogues, aza and deaza purine analogues, and other heterocyclic base analogues, wherein one or more of the carbon and nitrogen atoms of the purine and pyrimidine rings have been substituted by heteroatoms, e.g., oxygen, sulfur, selenium, phosphorus, and the like.

Detailed Description Text (12):
The terms "modified oligonucleotide polymer", "modified polynucleotide polymer" "modified polymer" and the like as used herein refers to oligonucleotides and/or polynucleotides with one or more chemical modifications at the molecular level of the natural molecular structures of all or any of the bases, sugar moieties, internucleoside phosphate linkages, as well as to molecules having added substituents, such as diamines, cholesterol or other lipophilic groups, or a combination of modifications at these sites. The internucleoside phosphate linkages can be phosphodiester, phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate and/or sulfone internucleotide linkages, or 3'-3', 5'-2' or 5'-5' linkages, and combinations of such similar linkages (to produce mixed backbone modified oligonucleotides). The modifications can be internal (single or repeated) or at the end(s) of the oligonucleotide molecule, and can include additions to the molecule of the internucleoside phosphate linkages, such as cholesteryl, diamine compounds with varying numbers of carbon residues between amino groups and terminal ribose, and deoxyribose and phosphate modifications which cleave or cross-link to the opposite chains or to associated enzymes or other proteins. Electrophilic groups such as ribose-dialdehyde could covalently link with an epsilon amino group of the lysyl-residue of such a protein. A nucleophilic group such as n-ethylmaleimide tethered to an oligomer could covalently attach to the 5' end of an mRNA or to another electrophilic site. The terms "modified oligonucleotide polymers", "modified polynucleotide polymers" and "modified polymers" also include oligonucleotides and/or polynucleotides comprising modifications to the sugar moieties (e.g., 2'-substituted ribonucleotide monomers), any of which are connected together via 5' to 3' linkages. Modified oligonucleotide polymers may also be comprised of PNA or morpholino modified backbones where target specificity of the sequence is maintained. A modified oligonucleotide polymer of the invention (1) does not have the structure of a naturally occurring oligonucleotide and (2) will hybridize to a natural nucleic acid, e.g., mRNA, or cDNA. Further, the modification preferably provides (3) higher binding affinity with RNA, (4) greater acid resistance, and (5) better stability against digestion with enzymes as compared to a natural oligonucleotide.

Detailed Description Text (14):
The term "end-blocked" as used herein refers to an oligonucleotide polymer with a chemical modification at the molecular level that prevents the degradation of selected nucleotides, e.g., by nuclease action. This chemical modification is positioned such that it protects the integral portion of the oligonucleotide, for example the region of the oligonucleotide that is targeted for hybridization (i.e., the test sequence of the oligonucleotide) or the portion of the oligonucleotide having a specific activity, e.g., enzymatic activity. An end block may be a 3' end block or a 5' end block. For example, a 3' end block may be at the 3'-most position of the molecule, or it may be internal to the 3' ends, provided it is 3' of the integral sequences of the oligonucleotide.

Detailed Description Text (15):
The term "substantially nuclease resistant" refers to oligonucleotide polymers that are resistant to nuclease degradation as compared to naturally occurring or unmodified oligonucleotides. Modified oligonucleotide polymers of the invention are at least 1.25 times more resistant to nuclease degradation than their unmodified counterpart, more preferably at least 2 times more resistant, even more preferably at least 5 times more resistant, and most preferably at least 10 times more resistant than their unmodified counterpart. Such substantially nuclease resistant oligonucleotide polymers include, but are not limited to, oligonucleotides with modified backbones such as phosphorothioates, methylphosphonates, ethylphosphotriesters, 2'-O-methylphosphorothioates, 2'-O-methyl-p-ethoxy ribonucleotides, 2'-O-alkyls, 2'-O-alkyl-n(O-alkyl), 3'-O-alkyls, 3'-O-alkyl-n(O-alkyl), 3'-O-methyl ribonucleotides, 2'-fluoros, 2'-deoxy-erythropentofuranosyls, 2'-O-methyl ribonucleosides, methyl carbamates, methyl carbonates, inverted bases (e.g., inverted

T's), or chimeric versions of these backbones.

Detailed Description Text (20):
The terms "associated oligonucleotide polymer," "associated polynucleotide polymer" and "substrate oligonucleotide polymer" and the like refer to the oligonucleotide or polynucleotide composition that makes up each of the samples associated to the array. Thus, the term "associated oligonucleotide polymer" includes oligonucleotide compositions of unique sequences and may include control or calibrating sequences (e.g., oligonucleotides corresponding to housekeeping genes). The oligonucleotide and/or polynucleotide compositions are preferably comprised of single stranded nucleic acid, where all of the modified nucleic acids in a sample composition may be identical to each other. Alternatively, there may be modified nucleic acids having two or more sequences in each composition, for example two different oligonucleotide polymers that are separate but complementary to each other.

Detailed Description Text (25):
In a preferred embodiment the polymers are oligonucleotides and/or polynucleotides with modified backbone structures, such as oligonucleotides with: 2'-F, 2'-O-alkyl and 2'-O-alkyl-n(O-alkyl), 3'-O-alkyl, and 3'-O-alkyl-n(O-alkyl) sugar moieties; changes in the ribose oxygen; 1' linkage modifications, 5' linkage modifications; and/or 3' linkage modifications. Modified oligonucleotides and polynucleotides of the invention also may be acid resistant and/or exonuclease resistant. In one embodiment, an exonuclease resistant block is added to the 3' or the 5' end of the oligonucleotide or polynucleotide depending on the attachment of the nucleic acid to the substrate. The resulting modified oligonucleotides and/or polynucleotides of the invention bind tightly to their RNA or DNA targets.

Detailed Description Text (27):
The modified oligonucleotide and/or polynucleotide polymers of the array may be synthesized to have approximately the same T.sub.m, by varying the length of the nucleic acids in each composition. Thus, an oligonucleotide polymer with an A-T rich sequence would be designed to be longer than an oligonucleotide polymer with a G-C rich sequence to provide approximately the same T.sub.m. The T.sub.m of each of the compositions on an array can be held relatively constant by providing lengths of oligonucleotides and/or polynucleotide polymers based on the binding affinity of the base sequence.

Detailed Description Text (28):
Acid stable associated oligonucleotide and/or polynucleotide polymers of the invention are stable when exposed to a pH of 1-2, while their binding partners are not. This allows an array having associated acid stable oligonucleotides and/or polynucleotide polymers to be exposed to a first sample, treated with an acidic solution applied in any of several possible protocols to free the array from the first binding partner, and reused with a second sample. Direct comparison of two different samples of binding partners using a single array has the advantage of limiting potential experimental variation present when comparing multiple arrays. Performing the experiment with the same sample on the same array allows a confirmation of the results obtained in the first instance, thus effectively confirming results without having variation in the array composition.

Detailed Description Text (29):
Similarly, associated end-blocked oligonucleotide and/or polynucleotide polymers display a resistance to nucleases, allowing the arrays to be exposed to DNA nucleases to free the array from a sample of binding partners. An array of the invention having nuclease resistant associated oligonucleotide polymers can be treated with an appropriate nuclease and reused with a different or the same sample.

Detailed Description Text (30):
The arrays of the present invention encompass associated polymers chemically modified to be acid stable from a pH of 0.01 to 7.0, and more preferably acid stable in a pH of 1.0 to 4.0, allowing such molecules to retain their structural integrities in acidic environments. Although a number of modifications are within the scope of the present invention, in a preferred embodiment the polymers of the invention are 2'-F, 2'-O-alkyl and 2'-O-alkyl-n(O-alkyl) modified oligonucleotides which, unlike unsubstituted phosphodiester or phosphorothioate DNA or RNA, exhibit significant acid

resistance in solutions with pH as low as 0-1 even at 37.degree. C. Acid stability of this first component coupled with the introduction of 3' and/or 5' acid stable, exonuclease resistant ends, confers several unique properties on the polymers of the invention. These low toxicity, highly specific, acid stable, end-blocked polymers represent a novel and improved oligonucleotide structure for use in array technologies.

Detailed Description Text (35):
In a preferred embodiment, the end-blocked oligonucleotide polymers of the devices and methods of the invention are substantially nuclease resistant and substantially acid resistant. This embodiment includes oligonucleotides completely or partially derivatized by one or more linkages from the group comprised of phosphorothioate linkages, 2'-O-methyl-phosphodiesters, 2'-O-alkyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-butyl, 2'-O-alkyl-n(O-alkyl), 2'-methoxyethoxy, 2'-fluoro, 2'-deoxy-erythropentofuranosyl, 3'-O-methyl, p-isopropyl oligonucleotides, phosphodiester, 2'-O(CH.sub.2 CH.sub.2 O).sub.x CH.sub.3, butyne, phosphotriester, phosphoramidate, propargyl, siloxane, carbonate, carboxymethylester, methoxyethoxy, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, methylphosphonate, phosphorodithioate, bridged phosphorothioate and/or sulfone internucleotide linkages, or 3'-3' or 5'-5' or 5'-2' linkages, and combinations of such similar linkages (to produce mixed backbone modified oligonucleotides), and any other backbone modifications.

Detailed Description Text (36):
Exemplary modifications that result in acid stability can be seen in FIGS. 1-6. End-blocked acid stable molecules are illustrated in FIGS. 7-8. Other modifications that may be of use in the present invention are illustrated. See "The Medicinal Chemistry of Oligonucleotides" in Medical Intelligence Unit: Therapeutic Applications of Oligonucleotides (1995) pp. 85-108; and Mesmaeker et al., Acc. Chem. Res., 28:366-374 (1995).

Detailed Description Text (38):
In a preferred embodiment, the oligonucleotide and/or polynucleotide polymer will have a backbone substantially resistant to acid degradation, exonuclease digestion, and endonuclease digestion. In the most preferred embodiment an oligonucleotide is uniformly modified, i.e., every base of the oligonucleotide is a 2'-O-alkyl, 2'-O-alkyl-n(O-alkyl), 3'-O-alkyl or 3'-O-alkyl-n(O-alkyl) modified base.

Detailed Description Text (40):
Oligonucleotides with a range of nuclease-resistant backbones were evaluated. As a result, preferred polymers of the present invention include end-blocked oligonucleotides with the chemical backbone structure of: 5'-biotin-2'-O-alkyl RNA-butanol-3'; 5'-butyl-2'-O alkyl RNA-biotin 3'; and 5'-butyl-2'-O-alkyl-biotin modified base-2'-O alkyl-butanol-3'(i.e., a biotin in the middle). This polymer readily associates with a substrate surface comprising avidin, strepavidin, and the like, and is both acid and nuclease resistant. Alternatively, an avidin, strepavidin or an avidin analog end-blocked modified nucleic acid (e.g., 5'-avidin-2'-O-alkyl RNA-butanol-3') can be associated to a solid support comprising biotin or biotin analog linkage groups. The end-blocking group on one end of the oligonucleotide may not be needed, depending on the manner of association with the substrate, as will be apparent to one skilled in the art upon reading the present disclosure.

Detailed Description Text (41):
Associated Oligonucleotide and Polynucleotide Compositions of the Arrays

Detailed Description Text (42):
Each associated modified oligonucleotide and/or polynucleotide composition of the pattern present on the surface of the substrate is preferably made up of a set of unique modified nucleic acids, and preferably a unique modified oligonucleotide and/or polynucleotide polymer composition. By "unique composition" is meant a collection or population of modified polymers capable of participating in a hybridization event under appropriate hybridization conditions, where each of the individual oligonucleotides may be the same (i.e., have the same nucleotide sequence) or different sequences, for example the oligonucleotide composition may consist of two different polymers that are complementary to each other (i.e., the two different

oligonucleotide polymers are complementary but physically separated so as to be single stranded, i.e., not hybridized to each other). In a preferred embodiment, the oligonucleotide compositions will comprise single stranded oligonucleotide polymers of one unique nucleotide sequence.

Detailed Description Text (43):
In those compositions having unique oligonucleotide polymers, the nucleotide sequence of the polymer is chosen in view of the type and the intended use of the array on which they are present. The unique oligonucleotide polymers are preferably chosen so that each distinct unique polymer does not cross-hybridize with any other distinct unique polymer on the array, i.e., the oligonucleotide polymer will not cross-hybridize to any other oligonucleotide compositions that correspond to a different gene. As such, the nucleotide sequence of each unique oligonucleotide polymer of a composition will have less than 90% homology, usually less than 85% homology, and more usually less than 80% homology with any other different associated oligonucleotide composition of the array, where homology is determined by sequence analysis comparison using the FASTA program using default settings. The sequence of unique associated oligonucleotide polymers in the compositions are not conserved sequences found in a number of different genes (at least two), where a conserved sequence is defined as a stretch of from about 4 to about 80 nucleotides which have at least about 90% sequence identity, where sequence identity is measured as above. The associated oligonucleotide polymers will generally have a length of from about 80 to about 300 nucleotides, usually from 100 to about 200 nucleotides. The length of the polymer can be chosen to best provide binding to the test sequence.

Detailed Description Text (44):
Although in a preferred embodiment the associated modified oligonucleotide composition will not cross-hybridize with any other associated oligonucleotide polymers on the array under standard hybridization conditions, associated oligonucleotide polymers and hybridization conditions can be altered to allow binding to multiple associated oligonucleotide compositions. For example, in determining the relatedness of a sample to oligonucleotides representing different members of a class of proteins, the polymer nucleotide sequences may be more similar and/or less stringent hybridization conditions may be used.

Detailed Description Text (48):
Although 2'-substituted oligonucleotides and polynucleotides exhibit marked acid stability and endonuclease resistance, they are sensitive to 3' exonucleases. In order to enhance the exonuclease resistance of 2'-substituted oligonucleotides and polynucleotides, the 3' or 5' and 3' ends of the oligoribonucleotide sequence are preferably attached to an exonuclease blocking function. For example, one or more phosphorothioate nucleotides can be placed at either end of the oligoribonucleotide. Additionally, one or more inverted bases can be placed on either end of the oligoribonucleotide, or one or more alkyls, e.g., butanol-substituted nucleotides or chemical groups, can be placed on one or more ends of the oligoribonucleotide. Other groups that can be put on include cholesterol, amino-groups, thiol-groups, glyceryl. Accordingly, a preferred embodiment of the present invention is an oligonucleotide comprising an oligonucleotide having the following structure:

Detailed Description Text (49):
wherein "B" is a 2'-F, 2'-O-alkyl or 2'-O-alkyl-n(O-alkyl) or 3'-O-alkyl or 3'-O-alkyl-n(O-alkyl) oligoribonucleotide between about 2 and about 300 bases in length, and "A" and "C" are respective 5' and 3' end blocking groups (e.g., one or more phosphorothioate nucleotides (but typically fewer than six), inverted base linkages, or alkyl, alkenyl, or alkynl groups or substituted nucleotides). A partial list of blocking groups includes inverted bases, dideoxynucleotides, methylphosphates, alkyl groups, aryl groups, cordycepin, cytosine arabanoside, phosphoramidates, a peptide linkage, dinitrophenyl group, 2'- or 3'-O-methyl bases with phosphorothioate linkages, 3'-O-methyl bases, fluorescein, cholesterol, biotin, biotin analogs, avidin, avidin analogs, strepavidin, acridine, rhodamine, psoralen, glyceryl, methyl phosphonates, butanol, butyl, hexanol, and 3'-O-alkyls. An enzyme-resistant butanol preferably has the structure HO--CH.sub.2 CH.sub.2 CH.sub.2 CH.sub.2 (4-hydroxybutyl) which is also referred to as a C4 spacer. An enzyme resistant butyl blocking group has the structure CH.sub.3 --CH.sub.2 --CH.sub.2 --CH.sub.2 --.

Detailed Description Text (50):
In a preferred embodiment, at least one end-block on the oligonucleotide is a biotin, biotin analog, avidin, or avidin analog. These molecules have the ability to both 1) block the degradation of the protected oligonucleotide or polynucleotide and 2) provide means for high affinity attachment of the modified nucleic acids to the solid support. Avidin and biotin derivatives which can be used to prepare the reagents of this invention include streptavidin, succinylated avidin, monomeric avidin, biocytin (biotin-.epsilon.-N-lysine), biocytin hydrazide, amine or sulfhydryl derivatives of 2-iminobiotin and biotinyl-.epsilon.-aminocaproic acid hydrazide. Additional biotin derivatives, such as biotin-N-hydroxysuccinimide ester, biotinyl-.epsilon.-aminocaproic acid-N-hydroxysuccinimide ester, sulfosuccinimidyl 6-(biotin amido)hexanoate, N-hydroxysuccinimideiminobiotin, biotinbromoacetylhydrazide, p-diazobenzoyl biocytin and 3-(N-maleimidopropionyl)biocytin, can also be used as end-blocking groups on the polynucleotides of the present invention.

Detailed Description Text (52):
The modified nucleic acids of the invention are associated to the surface of the solid support via a high affinity univalent or multivalent bonding. This binding is mediated by two binding units: 1) a binding unit on the modified oligonucleotide or polynucleotide polymer and 2) a binding unit associated on the surface of the solid support. In a preferred embodiment, the binding unit of the polymer is an end block, e.g. a 3' or 5' biotin or 3' or 5' avidin molecule. The affinity constant between the binding unit on the modified oligonucleotide and/or polynucleotide polymers and the binding units on the surface of the array will be greater than about 10.sup.7 M.sup.-1. More preferably, the K.sub.a will be greater than about 10.sup.11 M.sup.-1, and most preferably, the K.sub.a will be about 10.sup.15 M.sup.-1 or greater. The surface of the solid support may be evenly coated with the binding unit, (e.g., completely coated with a layer of avidin or an avidin analog if the nucleic acid molecule has a biotin or biotin analog, or completely coated with a layer of biotin or a biotin analog if the nucleic acid molecule contains avidin or an avidin analog), and the association of each polymer directed by the particular placement of each polymer. Alternatively, the binding units are attached directly to the array in preselected positions, and these positions define the subsequent positioning of the attached polymers.

Detailed Description Text (53):
One preferred embodiment of the present invention employs biotin or biotin analogs as the end-blocking binding units on the polymer. Typical biotin analogs include dethiobiotin, iminobiotin, 2-thiobiotin, azabiotin, biocytin, and biotin sulfone, bisnorazabiotin and other compounds readily apparent to one skilled in the art. Exemplary biotin analogs include, but are not limited by, those presented in Table 1. Biotin analogs include compounds and structures in which biotin is bound to another species. Exemplary biotin analogs can be found in U.S. Pat. Nos. 5,955,605, 5,247,081, 4,282,287, WO 97/29114, Green, "Avidin" in Advances in Protein Chemistry, Academic Press, vol. 29, 105 (1975); and Greg T. Hermanson, Bioconjugate Techniques, Academic Press (1996); Savage et al., Avidin Biotin Chemistry: A Handbook, Pierce Chemical Company (1992), all of which are incorporated by reference herein. The binding affinities of exemplary biotin analogs can be seen in Table 1.

Detailed Description Text (55):
Typical examples of avidin and avidin analogs include, but are not limited to, the avidin found in eggs, monomeric avidins, streptavidin, NeutrAvidin.TM. (Pierce Chemical Co). Streptavidin is a typical example of an avidin analog and is a bacterial biotin-binding protein which has physical characteristics similar to those of egg avidin, despite considerable differences in composition. Synthetic avidins, such as NeutrAvidin.TM., may have altered isoelectric points and non-specific binding compared to avidin, and thus may be preferable in certain instances as will be recognized by one skilled in the art upon reading this disclosure.

Detailed Description Text (64):
The modified polynucleotides and oligonucleotides that are associated on the array may also be produced using established techniques such as polymerase chain reaction (PCR) and reverse transcription (RT). These methods are similar to those currently known in the art (see e.g., Michael A. Innis (Editor), et al., PCR Strategies, (1995) and C. R.

Newton, A. Graham, PCR: Introduction to Biotechniques Series, (1997)), and preferably the enzymes used to produce the polynucleotides or oligonucleotides are optimized for incorporation of modified nucleotide monomers. Methods of identifying which enzymes are best suited for incorporation of nucleotide monomers with specific modifications (e.g., which enzymes will best incorporate 2'-modified dNTPs) are well known in the art, and thus one skilled in the art would be able to identify enzymes for use with the present invention based upon this disclosure. For example, the process of directed evolution can be used to unveil mechanisms of both thermal adaptation and incorporation efficiency and is an effective and efficient approach to identifying optimal enzyme activity. Multiple generations of random mutagenesis, recombination and high throughput can be used to create a polymerase that both incorporates modified nucleotide monomers, e.g., 2'-O-methyl substituted dNTPs, and remains thermostable at higher temperatures. See e.g., Zhao, H., et al. 12:47-53 (1999).

Detailed Description Text (65):
Biotin can be introduced into cDNAs by using biotinylated nucleotide triphosphates or biotinylated oligonucleotide primers. Likewise, cRNA can be generated by using biotinylated ribonucleotide triphosphates during synthesis of the RNA.

Detailed Description Text (71):
Although the invention is applicable to arrays of any type of polymer sequence the preferred arrays of the subject invention have a plurality of associated oligonucleotide and/or polynucleotide polymers stably associated with a surface of a solid support via the univalent or multivalent interactions of binding units. Each associated sample on the array comprises a modified oligonucleotide composition, of known identity, usually of known sequence, as described in greater detail below. Any conceivable substrate may be employed in the invention.

Detailed Description Text (72):
In the arrays of the invention, the modified oligonucleotide compositions are stably associated with the surface of a solid support, where the support may be a flexible or rigid solid support. By "stably associated" is meant that the sample of associated modified oligonucleotides and/or polynucleotide polymers maintain their position relative to the solid support under hybridization and washing conditions.

Detailed Description Text (81):
A single substrate supports more than about 10 different oligonucleotide and/or polynucleotide compositions and preferably more than about 100 different oligonucleotide and/or polynucleotide compositions, although in some embodiments more than about 10.sup.3, 10.sup.4, 10.sup.5, 10.sup.6, 10.sup.7, or 10.sup.8 different compositions are provided per cm.sup.2 of substrate surface. Of course, within a region of the substrate in which a modified oligonucleotide or polynucleotide polymer is attached, it is preferred that the modified nucleotides be substantially pure. In preferred embodiments, regions of the substrate contain oligonucleotide or polynucleotide polymers which are at least about 50%, preferably 80%, more preferably 90%, and even more preferably, 95% pure. Oligonucleotide or polynucleotide polymers having several sequences can be intentionally provided within a single region so as to provide an initial screening for biological activity, after which materials within regions exhibiting significant binding are further evaluated. In a preferred embodiment, each region will contain a substantially pure modified oligonucleotide or polynucleotide composition having a single sequence.

Detailed Description Text (83):
The amount of modified oligonucleotide or polynucleotide polymer present in each composition will be sufficient to provide for adequate hybridization and detection of nucleic acids during the assay in which the array is employed. Generally, the amount of oligonucleotide or polynucleotide in each composition will be at least about 0.1 ng, usually at least about 0.5 ng and more usually at least about 1 ng, where the amount may be as high as 1000 ng or higher, but will usually not exceed about 20 ng and more usually will not exceed about 10 ng. The copy number of each oligonucleotide or polynucleotide in a composition will be sufficient to provide enough hybridization sites to yield a detectable signal, and will generally range from about 0.01 fmol to 50 fmol, usually from about 0.05 fmol to 20 fmol and more usually from about 0.1 fmol to 5 fmol. Where the composition has an overall circular dimension, the diameter of the sample will generally range from about 10 to 5,000 .mu.m, usually from about 20 to

2,000 .mu.m and more usually from about 50 to 1000 .mu.m.

Detailed Description Text (84):
Control compositions may be present on the array including compositions comprising oligonucleotide or polynucleotide polymers corresponding to genomic DNA, housekeeping genes, negative and positive control genes, and the like. These latter types of compositions are not "unique" as that term is defined and used herein, i.e., they are "common." In other words, they are calibrating or control genes whose function is not to tell whether a particular "key" gene of interest is expressed, but rather to provide other useful information, such as background or basal level of expression. The percentage of samples which are made of unique nucleotide sequences that correspond to the same type of gene is generally at least about 30%, and usually at least about 60% and more usually at least about 80%.

Detailed Description Text (85):
With respect to the oligonucleotide and/or polynucleotide compositions that correspond to a particular type or kind of gene, type or kind can refer to a plurality of different characterizing features, where such features include: genes from or triggered by infectious organisms, such as bacteria and virus; function specific genes, where such genes include oncogenes, apoptosis genes, cytokines, receptors, protein kinases, etc.; genes specific for or involved in a particular biological process, such as apoptosis, differentiation, cell cycle regulation, cancer, aging, proliferation, etc.; location specific genes, where locations include organs, such as heart, liver, prostate, lung etc.; tissue, such as nerve, muscle, connective, etc.; cellular, such as axonal, lymphocytic, etc.; or subcellular locations, e.g., nucleus, endoplasmic reticulum, Golgi complex, endosome, lyosome, peroxisome, mitochondria, cytoplasm, cytoskeleton, plasma membrane, extracellular space; specific genes that change expression level over time, e.g., genes that are expressed at different levels during the progression of a disease condition, such as prostate genes which are induced or repressed during the progression of prostate cancer.

Detailed Description Text (87):
The length of the modified oligonucleotide polymers allows the compositions to bind with the same affinity to a RNA molecule as a much longer unmodified nucleic acid, e.g an unmodified cDNA. In the case where additional complementarity is needed to certain domains or regions found in a cDNA, multiple oligonucleotides may be used. Multiple oligonucleotide polymers directed at a particular gene or RNA molecule may be interspersed in a single region, or the different oligonucleotide polymers may each be in a discrete region, e.g., to determine presence or absence of related molecules in a sample.

Detailed Description Text (88):
As mentioned above, the arrays of the present invention typically comprise one or more additional associated oligonucleotide composition which serve as a control composition. In other words, the array may comprise one or more compositions that are made of non "unique" oligonucleotide polymers, e.g, modified polymers corresponding to commonly expressed genes. For example, compositions comprising modified polymers that bind to plasmid and bacteriophage nucleic acids, modified polymers which bind to genes from the same or another species which are not expressed and do not cross-hybridize with the test nucleic acid, and the like, may be present and serve as negative controls. In addition, compositions comprising housekeeping genes and other control genes from the same or another species may be present, e.g., to serve in the normalization of mRNA abundance and standardization of hybridization signal intensity in the sample assayed with the array.

Detailed Description Text (90):
In a preferred embodiment, the modified oligonucleotide polymers for use with the present invention are synthesized prior to attachment onto the substrate. This affords the advantages that: (1) oligonucleotide polymers of known composition and sequence can be produced; (2) oligonucleotide polymers can be analyzed and purified prior to attachment, which eliminates "shortmers," i.e., oligonucleotide polymers with insufficient length and/or incorrect sequence; (3) the methods used to produce oligonucleotides are less prone to error than current methods for production of cDNA, e.g,. PCR with Taq polymerase, (4) attachment to the substrate may be monitored or assayed without destroying the array and (5) the attachment is at a single tether

point.

Detailed Description Text (97):
Oligonucleotide polymers having a nucleotide sequence unique to that gene are preferably used in the present invention. Different methods may be employed to choose the specific region of the gene to be targeted. A rational design approach may also be employed to choose the optimal nucleotide sequence for the hybridization array. Preferably, the region of the gene that is selected is chosen based on the following criteria. First, the sequence that is chosen should yield an oligonucleotide polymer that preferably does not cross-hybridize with any other composition present on the array. Second, the sequence should be chosen such that the modified polymer has a low probability of cross-hybridizing with an nucleic acid having a nucleotide sequence found in any other gene, whether or not the gene is to be represented on the array. from the same species of origin, e.g., for a human array, the sequence will not be present in any other human genes. As such, sequences that are avoided include those found in: highly expressed gene products, structural RNAs, repeated sequences found in the sample to be tested with the array and sequences found in vectors. A further consideration is to select nucleotide sequences that provide for minimal or no secondary structure, structure which allows for optimal hybridization but low non-specific binding, equal or similar thermal stabilities, and optimal hybridization characteristics.

Detailed Description Text (99):
A variety of specific array types are also provided by the subject invention. As discussed above, array type refers to the nature of the oligonucleotide and/or polynucleotide compositions present on the array and the types of genes to which the associated compositions correspond. These array types include, but are not limited to: infectious organism array; human array; mouse array; developmental array; cancer array; apoptosis array; oncogene and tumor suppressor array; cell cycle gene array; cytokine and cytokine receptor array; growth factor and growth factor receptor array; neuroarrays; and the like.

Detailed Description Text (100):
In certain embodiments of the human array, human genes that may be represented by the composition on the arrays include those for: (a) oncogenes and tumor suppressors; (b) cell cycle regulators; (c) stress response proteins; (d) ion channel and transport proteins; (e) intracellular signal transduction modulators and effectors; (f) apoptosis-related proteins; (g) DNA synthesis, repair and recombination proteins; (h) transcription factors and general DNA binding proteins; (i) growth factor and chemokine receptors; (j) interleukin and interferon receptors; (k) hormone receptors; (l) neurotransmitter receptors; (m) cell surface antigens and cell adhesion proteins; (n) growth factors, cytokines and chemokines; (o) interleukins and interferons; (p) hormones; (q) extracellular matrix proteins; (r) cytoskeleton and motility proteins; (s) RNA processing and turnover proteins; (t) post-translational modification, trafficking and targeting proteins; (u) protein turnover; and (v) metabolic pathway proteins.

Detailed Description Text (109):
Following binding and visualization of a test sample on an array, the array may be treated to remove the bound test nucleic acids. The associated nucleic acid compositions remain intact following treatment, allowing reuse of the treated array. The array of the invention substantially retains its binding capabilities, and any differences in binding ability may be determined using control sequences associated on the array. Preferably, the array of the invention retains at least 75% of its binding capabilities, more preferably the array retains at least 85% of its binding capabilities, and even more preferably the array of the invention retains at least 95% of its binding capabilities.

Detailed Description Text (110):
Arrays with associated modified oligonucleotide and/or polynucleotide compositions can be exposed to a low pH environment, e.g., pH from 0.5-4.5, which results in the degradation of non-modified nucleic acids. Following the treatment, the arrays of the invention are rinsed to remove any unwanted test nucleic acid fragments, residual label and the like, and the arrays are prepared for reuse.

Detailed Description Text (124):
Kits Having Arrays of Present Invention

Detailed Description Text (125):
Also covered are kits for performing analyte binding assays using the arrays of the present invention. Such kits according to the subject invention will at least comprise the arrays of the invention having associated modified polymers of the present invention. Kits also preferably comprise an agent for removal of test binding agents, e.g., a solution with low pH and/or with nuclease activity. The kits may further comprise one or more additional reagents employed in the various methods, such as: 1) primers for generating test nucleic acids; 2) dNTPs and/or rNTPs (either premixed or separate), optionally with one or more uniquely labeled dNTPs and/or rNTPs (e.g., biotinylated or Cy3 or Cy5 tagged dNTPs); 3) post synthesis labeling reagents, such as chemically active derivatives of fluorescent dyes; 4) enzymes, such as reverse transcriptases, DNA polymerases, and the like; 5) various buffer mediums, e.g., hybridization and washing buffers; 6) labeled probe purification reagents and components, like spin columns, etc.; and 7) signal generation and detection reagents, e.g., streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like.

Detailed Description Text (134):
The molecules can be biotinylated at any time during production of the modified oligonucleotide depending upon where the attachment site is desired. For example: 3' attachment of a modified oligonucleotide can be achieved using 3' biotin CPGs to attach the biotin to the 3' end of the molecule; during synthesis a 5'-biotin nucleotide amidite can be incorporated to the end of the molecule to allow 5' attachment; and a biotin-nucleotide amidite can be incorporated into the molecule at any desired position for attachment in the center of the molecule.

Detailed Description Text (141):
Homopolymers of 2'-O-methyl A, C, G, and U twelve bases long, were synthesized with 3' and 5' inverted T-blocked ends. They were purified, desalted, lyophilized, and dissolved at 300 A.sub.260 per ml in sterile water. Samples were removed and diluted 1 to 4 with either 0.1 N HCl or 1.0 N HCl to give final pHs of approximately 1 and 0, respectively, and placed in a heat block at 39.degree. C. Aliquots were taken at 0, 2, 4 and 24 hours, diluted 1:20 into a solution of 0.025 M NaOH and 0.03 M NaCl, stored at -20.degree. C. until being run on an analytical HPLC under strongly denaturing conditions on an anion exchange column.

Detailed Description Text (145):
A 14 mer heteropolymer was synthesized as a regular phosphodiester DNA (O), a phosphorothioate DNA (S), an unblocked 2'-O-methyl RNA (2' om), a 2'-O-methyl RNA with 3' and 5' butanol blocked ends (B2' om), and a phosphorothioate chimera having four 2'-O-methyl phosphorothioate bases on either side of 6 interior phosphorothioate DNA bases (SD). They were purified, desalted, lyophilized, and dissolved at 300 A.sub.260 per ml in sterile water. Samples were removed and diluted 1 to 4 with 0.1 N HCl to give a final pH of approximately 1.5, and placed in a heat block at 39.degree. C. Aliquots were taken at the times indicated and diluted 1:20 into a solution of 0.025 M NaOH and 0.03 M NaCl, and were run on an analytical HPLC under strongly denaturing conditions on an anion exchange column. Initially all but the end-blocked 2'-O-methyl RNA solutions became cloudy upon addition of the HCl. Upon heating, both the phosphodiester DNA and the unblocked 2'-O-methyl RNA became clear. The two oligonucleotides with phosphorothioate linkages appeared cloudy until about 2 hours when they slowly began to clear as they decomposed.

Detailed Description Text (149):
A 14 mer heteropolymer was synthesized as a regular phosphodiester DNA, a phosphorothioate DNA, an unblocked 2'-O-methyl RNA, and a 2'-O-methyl RNA with 3' and 5' butanol blocked ends. They were purified, desalted, lyophilized, and dissolved at 300 A.sub.260 per ml in sterile water. Samples were removed, diluted into human serum (Sigma, H 2520), and incubated at 37.degree. C. Aliquots were taken at 2 and 4 days and diluted and filtered before being run on an analytical HPLC under strongly denaturing conditions on an anion exchange column.

Detailed Description Text (150):

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

Detailed Description Text (153):
A 2'-OMe RNA oligo 150-mer (SEQ ID NO:3) was captured using a 15-mer 2'-OMe oligo tethered through biotin-streptavidin onto a white 96-well plate. Observations were taken of individual 2'-OMe 12-mers complementary to portions of the 150-mer labeled on the 5'-end with Oregon Green 488. Once the signal levels were obtained for single sequences, various combinations of the 12-mers were used to determine whether the fluorescence signal from the probes was additive. The sequences of these probes were chosen at 12-base steps through the sequence of the 150-mer. Results were as follows:

Detailed Description Text (155):
While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

Detailed Description Paragraph Table (6):
% of Full Length Oligo Polymer t = 0 2d 4d Phosphodiester-DNA 100 65 35 Unblocked 2'-O-methyl RNA 100 87 72 End-Blocked 2'-O-methyl RNA 100 100 100 Phosphorothioate 100 100 100

CLAIMS:

1. An array comprising a plurality of modified oligonucleotide compositions stably associated with a surface of a support, comprising: a support comprised of a plurality of distinct areas on a surface of the support, said areas having a plurality of a first binding unit bound to the surface; and a plurality of different modified oligonucleotide compositions wherein each composition is characterized by an oligonucleotide backbone structure modified from that of a naturally occurring nucleotide polymer, wherein oligonucleotides of the compositions comprise a second binding unit which forms a non-covalent bond with said first binding unit and a blocking chemical modification at or near at least one end of each oligonucleotide; wherein each different modified oligonucleotide composition is stably associated with a different distinct area of the support, and further wherein modified oligonucleotides of each composition are characterized by a pH stability of at least one hour at 37.degree. C. at a pH range of about 0.5 to about 6.0 and a nuclease resistance of at least twice that of a naturally occurring oligonucleotide having the same sequence and number of bases.

12. The array of claim 1, wherein each modified oligonucleotide composition on each distinct area comprises a population of identical oligonucleotides.

13. The array of claim 1, wherein the number of modified oligonucleotide compositions on said array ranges from about 2 to about 10.sup.9.

14. A method of synthesizing an array comprising a plurality of modified oligonucleotide compositions stably associated with a surface of a support, said method comprising: synthesizing a support comprising a plurality of distinct areas having a plurality of a first binding unit bound to the surface; synthesizing a plurality of different modified oligonucleotide compositions wherein each composition comprises: an oligonucleotide backbone structure modified from that of a naturally occurring nucleotide polymer, and a second binding unit which forms a non-covalent bond with said first binding unit and a blocking chemical modification at or near at least one end of each oligonucleotide; wherein the modified oligonucleotides of each composition are characterized by a pH stability of at least one hour at 37.degree. C.

at a pH range of about 0.5 to about 6.0 and a nuclease resistance of at least twice that of a naturally occurring oligonucleotide having the same sequence and number of bases; and stably associating each modified oligonucleotide composition to a distinct area of said support.

15. The method of claim 14, wherein the second binding unit is associated to the modified oligonucleotides of each composition by a linker molecule.

16. The method of claim 14, wherein the second binding unit is incorporated into the modified oligonucleotides of each composition during synthesis.

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